

REMARKS

Following entry of the foregoing amendments, claims 5-7, 11-18, 20-21, 23, and 26-42 constitute the pending claims in the present application. Claims 1-4, 8-10, 19, 22, and 24-25 are cancelled. Claims 20 and 21 are withdrawn. Claims 29-42 are newly added.

Introductory Matters

Applicants bring to the Examiner's attention that the claims presented herewith are identical to those presented with Applicants Reply to the Final Office Action, mailed November 15, 2005 (not entered). Applicants are elaborating their argument in support of amending the claims to include a complexing agent comprising "...at least one host/guest moiety at a terminus of the complexing agent..." and highlight additional support in the specification for said amendments (*vide infra*). Applicants are also elaborating their argument in support of patentability over US 6,740,643 to Wolff et al.

Applicants hereby amend claim 5 to more particularly define the present invention. In particular, Applicants amend claim 5 to recite that the complexing agent comprises "...at least one host/guest moiety at a terminus of the complexing agent..." Support for this amendment can be found in the specification. For example, on page 42, line 14, a general formula of a complexing agent pictorially depicts that the host/guest moiety is at the terminus of the complexing agent. Additionally, on page 43, line 18, another embodiment of the complexing agent is shown in a different general formula where again the host/guest moiety is at the terminus of the complexing agent. Moreover, in Examples 22-35, 40-42, 44-65, specific embodiments of complexing agents wherein the host/guest moiety is at the terminus of the complexing agent are exemplified. Hence, the specification discloses both in general terms and in specific embodiments that the complexing agent may comprise a host/guest moiety at a terminus. As such, Applicants submit that this subject matter is contemplated by the present invention and adequately supported by the specification as originally filed.

Applicants further amend Claim 5 to recite that the complexing agent comprises "...at least one polymer portion that increases solubility and/or imparts stabilization relative to a composition of the cyclodextrin-containing polymer and therapeutic agent alone..." Support for

BEST AVAILABLE COPY

this amendment can be found in claims 15 and 16 as well as in the specification on page 41, lines 2-4.

Claims 14-17 are amended to correct matters of form. Claim 27 is also amended to correct matters of form and to address an outstanding rejection (*vide infra*).

Subject matter from claim 22 is incorporated into new claim 30 (*vide infra*), and claim 22 is hereby cancelled. Claims 24-25 are also hereby cancelled.

Applicants submit the above amendments present no new matter and reserve the right to pursue similar subject matter to the unamended and/or cancelled claims in subsequent divisional and/or continuation applications.

Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action.

Telephonic Interview and Advisory Action

Applicants appreciate the Examiner's time and attention in the telephonic interview of October 21, 2005. Applicants also appreciate the Examiner's clarification in the Advisory Action of December 8, 2005.

1. Claim Rejections – 35 USC 112, First Paragraph

The Examiner has rejected claims 5-7, 11-18, 21-22, 24-25, and 27-28 under 35 USC 112, first paragraph as failing to comply with the written description requirement contending that the claims contain subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that Applicants had possession of the invention at the time the application was filed. In particular, the Office asserts that "polymeric portion," as recited in claim 5, is broader than what is described in the specification.

As mentioned above, Applicants are amending claim 5 to recite that the polymer portion "...increases solubility and/or imparts stabilization relative to a composition of the cyclodextrin-containing polymer and therapeutic agent alone..." Applicants submit that claim 5 as currently

amended is adequately supported by the specification. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

2. *Claim Rejections – 35 USC 112 Second Paragraph*

The Examiner has rejected claim 27 under 35 USC 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Specifically, the Examiner notes that there is insufficient antecedent basis for “the side chains” in claim 27. Applicants are amending claim 27 to address the instant rejection, and as amended claim 27 no longer recites “*the* side chains.” Consequently, Applicants respectfully request reconsideration and withdrawal of the rejection.

3. *Claim Rejections – 35 USC 102(e)*

Claims 5-7, 12-18, and 22-27 are rejected under 35 USC 102(e) as allegedly being anticipated by US 6,740,643 to Wolff et al. (“Wolff”). The Office states that “Wolff discloses several compositions comprising a cyclodextrin-containing polymer, plasmid DNA and a complexing agent...Example 6 comprises a composition including a polymer containing cyclodextrins in the side chains, plasmid DNA and Triton-X 100 (a PEG ether of octylphenol).” Applicants respectfully traverse the rejection to the extent that it is maintained over the claims as currently amended.

Applicants assert that Wolff does not teach or suggest all the limitations of the pending claims as currently amended and, thus, does not anticipate the instant claims. In particular, Wolff does not teach or suggest a complexing agent comprising “...at least one host/guest moiety at a terminus of the complexing agent...” as recited in claim 5 as currently amended. Applicants are submitting Exhibit A, a print out from the Roche Applied Science website where Triton X-100 is offered for sale (<http://www.roche-applied-science.com/pack-insert/1332481a.pdf>), and Exhibit B, a copy of the article Tang et al. *Analyst*, 2005, 130, 1038-1045. Exhibit A shows the structure of Triton X-100. Of particular importance is that the phenyl moiety of Triton X-100 links the PEG chain and the octyl moiety; that is, the phenyl moiety of Triton X-100 is between the PEG chain and the octyl moiety and is *not at a terminus*. As shown in Scheme 3 of Exhibit B, it is the phenyl moiety of Triton X-100 that participates in host/guest interactions with beta-cyclodextrin. Thus, since the phenyl moiety of Triton X-100

undergoes host/guest interactions with beta-cyclodextrin, and since the phenyl moiety is not at a terminus, it is clear that Wolff does not teach or suggest a complexing agent comprising "...at least one host/guest moiety at a terminus of the complexing agent..." as recited in claim 5 as currently amended.

Additionally, regarding claims 12 and 13, the Triton X-100 of Wolff does not fall within the formulas of the claims. In particular, the formulas of claims 12 and 13 each depict a host/guest moiety at a terminus of the structure. As noted above, Triton X-100 does not possess a terminal host/guest moiety, since the phenyl moiety is not at a terminus.

As such, Wolff does not teach all the limitations of the pending claims as currently amended and does not anticipate the instant claims. Accordingly, Applicants request reconsideration and withdrawal of the rejection.

4. *Claim Rejections – 35 USC 103(a)*

Claims 5-7, 12-18, and 22-28 are rejected under 35 USC 103(a) as allegedly being obvious over Wolff. Applicants respectfully traverse the rejection to the extent that it is maintained over the claims as currently amended. Pursuant to MPEP 2142, “[t]o establish a *prima facie* case of obviousness...the prior art reference (or references when combined) must teach or suggest all the claim limitations.” *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). As noted above, Wolff does not teach all the limitations of the pending claims as currently amended, in particular, a complexing agent comprising "...at least one host/guest moiety at a terminus of the complexing agent," and, hence, there is no apparent suggestion of the particular combination of features recited in claim 5 as amended.

Regarding claims 12 and 13, as noted above, Wolff does not teach all the limitations of these claims, since Wolff does not disclose a host/guest moiety at a terminus of a complexing agent as depicted in the formulas of claims 12 and 13.

Furthermore, there is no motivation provided by Wolff and/or the knowledge in the art at the time of filing, to modify the teachings of Wolff to arrive at the present claims. Considering that Wolff does not teach all the limitations of the present claims and the lack of motivation to

modify Wolff to arrive at said claims, Applicants assert that the claims are not rendered obvious by Wolff. Accordingly, Applicants request reconsideration and withdrawal of the rejection.

5. Double Patenting Rejection

Claims 5-7 and 11-21 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 14-20 of U.S. Patent Application No. 10/021,312. Applicants will submit a terminal disclaimer, if necessary, upon indication of allowability.

Newly Added Claims

Claims 29-42 are newly added. Support for claim 29 can be found in claims 15-16 as well as in the specification on page 41, lines 2-4. Claim 30 recites similar subject matter as unamended claim 5 with the additional limitation that "...the complexing agent comprises at least one polymeric spacer group..." Support for claim 30 can be found in claims 5, 18, and 22 as well as in specification from page 41, line 24 to page 42, line 7. New claims 31-42, which depend directly or indirectly from claim 30, include subject matter from existing claims according to the following table:

New Claim	Existing Claim
30-32	5-7
33	11
34	23
35-40	12-17
41-42	27-28

Applicants assert that claims 29-42 present no new matter.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Should an extension of time be required, Applicants hereby petition for same

and request that the extension fee and any other fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945**.

Respectfully Submitted,

Date: January 17, 2006



David P. Halstead, Ph.D.
Reg. No. 44,735

Customer No: 28120
Fish & Neave IP Group
Ropes & Gray LLP
One International Place
Boston, MA 02110
Phone: 617-951-7000
Fax: 617-951-7050

Exhibit A

For life science research only. Not for use in diagnostic procedures.
FOR *IN VITRO* USE ONLY.

Triton X-100

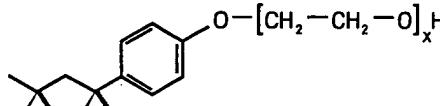
Octylphenolpoly(ethyleneglycoether)_x
Especially purified for membrane research

Cat. No. 1 332 481

50 ml (5 x 10 ml)

Version 3, Sept. 2003

Store at 2-8° C

Detergent type	Non-ionic detergent	Absorbance (254 nm): 0.88 (0.2% w/v) (278 nm): 1.11 (0.5% w/v)
Structure		Cloud point 65° C
Formula	C ₃₄ H ₆₂ O ₁₁ for x = 10	Working concentration* > 1-5 mM (1, 2)
Molecular weight	647 for x = 10	Note Membrane proteins are highly sensitive towards peroxides and carbonyl compounds. While proteins are oxidized by peroxides, the Schiff's base formed during the reaction with carbonyl compounds can for example influence the proteins function. Also salts are disturbing the isolation of membrane bound proteins. Detergents of the polyoxyethylene type might contain - depending on production and storage - contaminations of peroxides, carbonyl compounds and salts. Peroxide formation is strongly enhanced by light (3, 4). Triton X-100 has been purified to reduce levels of unwanted peroxides, carbonyl compounds and salts. The filling in airtight injection vials guarantees the constant quality during dispatch and storage. Roche Diagnostics supplies all detergent solutions of the polyoxyethylene type subdivided in 10 ml vials to prevent formation peroxide after the first use. The solutions can easily be removed through the cap of the vial with a syringe.
Form	10% aqueous solution (w/v), filled in injection bottles under nitrogen.	
Typical analysis	Peroxide content (as H ₂ O ₂) < 2 ppm	
Application	Triton ¹⁾ X-100 is one of the most commonly used non-ionic detergents for solubilizing membrane proteins during isolation of membrane-protein complexes.	References
Critical micelle concentration (CMC)	approx. 0.2 × 10 ⁻³ M (25° C)	<ol style="list-style-type: none">1 Barbero, M. L. et al. (1984) <i>Arch. Biochem. Biophys.</i> 228, 560-568.2 Schubert, D. et al. (1983) <i>FEBS Lett.</i> 163, 81.3 Ashanti, Y. C. & Catravas, G. N. (1980) <i>Anal. Biochem.</i> 109, 55-62.4 Lever, M. (1977) <i>Anal. Biochem.</i> 83, 274-284.
Stability	Stable at 2-8°C, protected from light.	

Related products, available from Roche Diagnostics.

Product	Cat. No.	Pack size
Detergent Adsorber-Gel	1 500 678	50 ml
Non-Ionic Detergents		
Digitonin, high purity	1 500 643	1 g
	1 518 968	5 g
n-Dodecyl- β -maltoside	821 608	1 g
Nonidet ²⁾ P40	1 332 473	5 x 10 ml
aqueous solution, 10% w/v		
n-Octylglucoside	411 434	1 g
	737 062	2 g
	1 585 401	5 g
	634 425	10 g
	737 674	20 g
	1 359 088	50 g
Thesit ³⁾	836 630	100 g
Triton X-100	789 704	100 ml
Triton X-114	1 033 441	100 ml
Tween ⁴⁾ 20	1 332 465	5 x 10 ml
aqueous solution, 10% w/v		
Ionic Detergents		
Deoxycholic acid	1 332 597	5 g
Sodium dodecyl sulfate (SDS)	1 028 685	100 g
	1 028 693	500 g
Zwitterionic Detergents		
CHAPS	810 100	1 g
	810 118	10 g
	810 126	50 g
N-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate	1 112 724	50 g
Detergent Trial Set	1 652 826	1 set
Digitonin (high purity), Dodecyl- β -maltoside, Octylglucoside, Nonidet P40, Triton X-100, Tween 20, Deoxycholic acid and CHAPS.		

This combination comprises only the most important products related to the product described. Please refer to our latest catalogue for our current product range or contact your local RAS representative directly.

* We recommend to use these values only as a starting point. The optimal working concentration can differ depending on the application.

¹⁾ Triton is a trademark of Rohm & Haas Company, Philadelphia, PA, USA.

²⁾ Nonidet is a trademark of Shell International Petroleum Company Limited, U.K.

³⁾ Thesit is a trademark of Desitin-Werk, Carl Klinke GmbH, Hamburg, Germany.

⁴⁾ Tween is a trademark of ICI Americas, Inc., USA.

How to contact Roche Applied Science

www.roche-applied-science.com

to order, solve technical queries, find product information, or contact your local sales representative.

www.roche-applied-science.com/pack-insert/1332481a.pdf

Please visit our new Online Technical Support Site



Roche Diagnostics GmbH
Roche Applied Science
Nonnenwald 2
82372 Penzberg
Germany

Highly sensitive and selective room-temperature phosphorescence determination of thiabendazole by the supramolecular interaction of thiabendazole/β-cyclodextrin/triton X-100

Bo Tang,* Xu Wang, Guangli Wang, Yan Wang and Zhenzhen Chen

Received 7th March 2005, Accepted 9th May 2005

First published as an Advance Article on the web 9th June 2005

DOI: 10.1039/b503217b

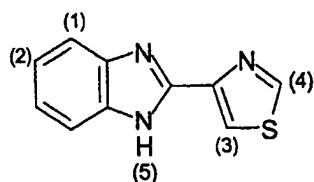
A strong and stable room temperature phosphorescence (RTP) signal ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 298/481 \text{ nm}$) resulting from a 1 : 1 : 1 β -cyclodextrin (β -CD)/thiabendazole (TBZ)/triton X-100 (TX-100) supramolecular ternary inclusion complex was induced by KI as a heavy atom perturber. Based on the heavy-atom induced RTP, a new phosphorescence method for TBZ determination was established. The analytical curve of TBZ gave a linear range of 20–820 ng mL⁻¹ with a detection limit and relative standard deviation of 2.1 ng mL⁻¹ and 1.9%, respectively. The interference of 46 coexisting substances was studied. Compared with the method using a chemical oxygen scavenger, this method is simpler as deoxygenation of the solution is not required. The detection limit and the heavy-atom concentration of the proposed method were decreased about 8 and 4 times, respectively. The lifetime of the phosphorescence was prolonged 9 times and the pH range was greatly broadened. The proposed method has been successfully applied to the determination of TBZ in tap water, lake water and pineapples.

1 Introduction

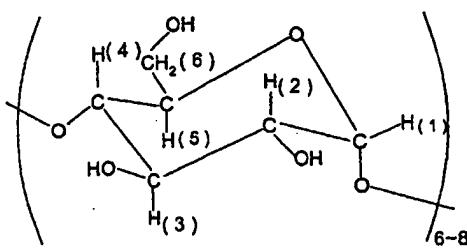
Thiabendazole [2-(4-thiazolyl)-1*H*-benzimidazole, TBZ, Scheme 1] is a broad-spectrum bactericide used for human and domestic animals.¹ It also can be used as food additives,² a fresh keeper for vegetables and fruits,³ a pre- or post-harvest fungicide⁴ and an industry mildewproof agent. In view of its wide applications, some methods have been established for its quantification determination, for example, high performance liquid chromatography (HPLC),⁵ liquid chromatography mass spectrometry (LC-MS),⁶ fluorimetry,⁷ capillary electrophoresis,⁸ room temperature phosphorimetry (RTP),⁹ micelle electrokinetic capillary chromatography (MEKC),¹⁰ capillary atomization mass spectrometry,¹¹ capillary zone electrophoresis (CZE),¹² and liquid chromatography-tandem mass spectrometry (LC-MS-MS)¹³ have been employed to determine TBZ in a variety of matrices such as serum, plasma, urine, saliva, pharmaceutical preparations, pesticide residues and animal bodies.

Cyclodextrins (CDs), the cyclic oligosaccharides consisting of six or more D-(+)-glucopyranose units (Scheme 2), are well known to have a hollow truncated cone with a hydrophobic cavity and hydrophilic wall to form inclusion complexes with organic or inorganic guest molecules which possess suitable polarity and dimension. As an excellent enzyme model and

molecule receptor, CDs has been widely used in the fields of science and technology.¹⁴ Because the formation of supramolecular complex with CDs can alter the photochemical and photophysical properties of the guest molecules, considerable attention has been focused on the luminescence application of the CDs. Drug molecules can demonstrate dramatically different physical, chemical and biological properties through formation of inclusion complexes with CDs,¹⁵ such as the enhancement of the solubility, stability and bioavailability.¹⁶ Surfactants can provide a similar microenvironment like the CDs,¹⁷ which can enhance solubility, stability, and sensitivity of analysis systems. Some chemical and physical properties of surfactants can be changed when they are included in the cavity of CDs, so the interaction of surfactants with CDs has attracted much attention recently.¹⁸ Studies on the formation of multirecognition ternary CD complexes in which two different guests are complexed in a single CD host cavity, have been performed.¹⁹ In a drug/CD/surfactant multirecognition ternary complex, drug molecules can show remarkably different spectral characteristics from those in a binary drug/CD complex, which leads to very high sensitivity and selectivity of the analytical method, so this method has a very important theoretical and applicative value.



Scheme 1 Chemical structure of TBZ.



Recently, Blanco *et al.* reported the room temperature phosphorescence (RTP) spectral parameters of some polycyclic aromatic hydrocarbons and nitrogen heterocycles (including TBZ) in water solution without any protective medium.⁹ This new type of RTP emission was named as non-protective fluid RTP²⁰ (NP-RTP) or heavy-atom induced RTP²¹ (HAI-RTP). However, in the report, optimizations of the chemical variables for the determination of TBZ were not satisfactory. For example, the concentration of the heavy atom perturber, KI, was as high as 0.8 mol L^{-1} ; the phosphorescence lifetime of TBZ was only $89 \mu\text{s}$ and the pH range was limited to 6.5–10 because of the addition of chemical deoxygenator-sodium sulfite. Therefore, the sensitivity of the method was low and the experimental conditions were difficult to control. In the present work, intense and stable RTP from a TBZ/TX-100/β-CD ternary inclusion complex was observed by using KI as the heavy atom perturber. Based on the reaction, a novel phosphorescence method for the determination of TBZ was established. Compared with the reported HAI-RTP method,⁹ the proposed method need not use a chemical oxygen scavenger; the detection limit and the heavy-atom concentration was lowered about 8 and 4 times, respectively. The lifetime of the phosphorescence was prolonged 9 times; the pH range was greatly broadened. The phosphorescence intensity I_p was linear over a TBZ concentration range of $20\text{--}820 \text{ ng mL}^{-1}$. The detection limit and relative standard deviation was 2.1 ng mL^{-1} and 1.9%, respectively. The interference of 46 coexisting substances was slight. Moreover, this method is simpler as deoxygenation of the solution is not required. The proposed method has been successfully applied to the determination of TBZ in tap water, lake water and pineapples.

2 Experimental

2.1 Instruments and chemicals

All steady-state luminescence spectra were carried out on a Perkin-Elmer (Norwalk, CT, USA) LS-5 luminometer, equipped with a xenon lamp, 1.0 cm quartz cells and a Perkin-Elmer Model 561 recorder, phosphorescent mode, $t_d/t_g = 0.15/0.25 \text{ ms}$, $\text{slits}_{\text{ex/em}} = 15/20 \text{ nm}$. Infrared spectra were obtained from a PE-983G IR-spectrophotometer (KBr, discs, Norwalk, CT, USA). All pH measurements were made with a pH-3C digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. The sample chamber accommodated a thermostated cuvette holder, controlled to $25 \pm 1 \text{ }^\circ\text{C}$ via a CS-50 constant temperature circulator (Chongqing Experimental Equipment Works, Chongqing, China). Surface tension measurements were made using an ST-1 surface tensiometer (Shimadzu Co., Japan).

TBZ (purchased from China Medicine (Group) Shanghai Chemical Reagent Corporation, Shanghai, China) was of analytical reagent grade. Its stock solution ($2.0 \times 10^{-5} \text{ g mL}^{-1}$) was prepared in acetonitrile and stored in the dark in amber bottles at $4 \text{ }^\circ\text{C}$, diluted into $5.0 \times 10^{-6} \text{ g mL}^{-1}$ when used. β-CD (obtained from China Medicine (Group) Shanghai Chemical Reagent Corporation, Shanghai, China) was purified by twice recrystallization in double-distilled water, followed by vacuum drying at $60 \text{ }^\circ\text{C}$ for 12 h and used with a concentration

analytical reagent grade and used with a concentration of $3.0 \times 10^{-3} \text{ mol L}^{-1}$. KI (obtained from Tianjin Hai Hua Fine Chemical Works, Tianjin, China) was analytically pure and used with a concentration of 2.00 mol L^{-1} . A sodium citrate-sodium hydroxide buffer solution (0.10 mol L^{-1} , pH = 5.50) was used. Other chemicals used were of analytical reagent or higher grade. Doubly distilled water was used throughout.

2.2 Experimental procedure

Into a 10 mL colorimetric tube were added 2.00 mL of sodium citrate-sodium hydroxide buffer solution, 0–4 mL TBZ stock solution (in the concentration range of $20\text{--}820 \text{ ng mL}^{-1}$), 1.00 mL of $5.0 \times 10^{-3} \text{ mol L}^{-1}$ β-CD, 2.00 mL of $3.0 \times 10^{-3} \text{ mol L}^{-1}$ TX-100 and 1.00 mL of 2 mol L^{-1} KI sequentially. The mixed solution was diluted to 10 mL with doubly distilled water and allowed to equilibrate at $20 \pm 1 \text{ }^\circ\text{C}$ for 10 min. Then the phosphorescence intensity was measured at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 298/481 \text{ nm}$ with $t_d/t_g = 0.15/0.25 \text{ ms}$ against a reagent blank. This procedure was replicated in order to obtain three or more phosphorescence intensity values for each of the TBZ concentrations studied. A calibration graph was prepared under the same conditions for the determination of TBZ.

Surface tension measurements were made using the hanging slice method²² at $25 \pm 1 \text{ }^\circ\text{C}$. About 30–40 measurements were made for a particular solution and the standard deviation of surface tension from the mean value was found to be less than $\pm 0.2 \text{ mN m}^{-1}$.

3 Results and discussion

3.1 RTP spectra

Fig. 1 shows the phosphorescence spectra of TBZ in different media. As can be seen, in the presence of only β-CD or

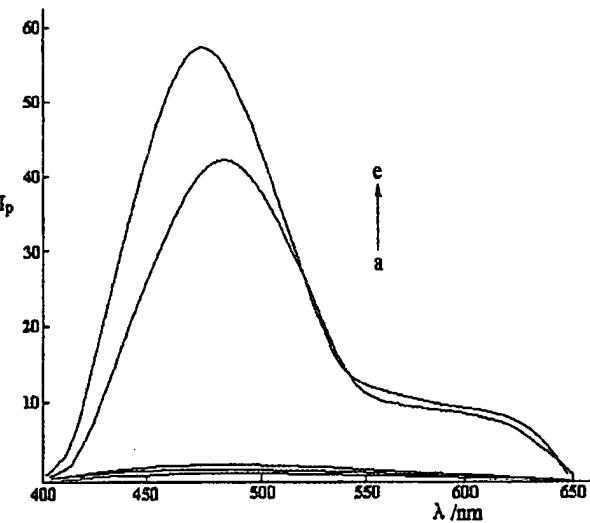


Fig. 1 Room temperature phosphorescence (RTP) spectrum of TBZ. a: β-CD/TBZ/KI, b: TX-100/TBZ/KI, c: β-CD/TBZ/TX-100, d: TBZ/Na₂SO₃/KI, e: β-CD/TBZ/TX-100+KI. a, b, c, e: $C_{\text{TBZ}} = 200 \text{ } \mu\text{g L}^{-1}$, $C_{\beta\text{-CD}} = 5.0 \times 10^{-4} \text{ mol L}^{-1}$, $C_{\text{TX-100}} = 6.0 \times 10^{-4} \text{ mol L}^{-1}$, $C_{\text{KI}} = 0.20 \text{ mol L}^{-1}$, pH = 5.50; d: $C_{\text{TBZ}} = 200 \text{ } \mu\text{g L}^{-1}$,

TX-100, no phosphorescence is observed, which indicates that the protective shielding effect resulting solely from an apolar or ordered microenvironment can not dramatically increase the $S_1 \rightarrow T_1$ intersystem crossing efficiencies in this experiment. In the β -CD/TBZ/KI (Fig. 1a, $\lambda_{ex}/\lambda_{em} = 301/482$ nm) or TX-100/TBZ/KI (Fig. 1b, $\lambda_{ex}/\lambda_{em} = 297/490$ nm) system, weak phosphorescence is observed, which suggests that although the heavy atom perturber KI can induce the $S_1 \rightarrow T_1$ intersystem crossing rates of TBZ to increase, the ternary inclusion complex is not formed. The rigidity of the system is not strong enough to shield against light, oxygen and heat, so the non-radiative decay of TBZ, such as the dissolved oxygen decay effect, collisions with solvent, conversion of energy etc., deactivate its excited triple state and the $T_1 \rightarrow S_0$ non-radiative transition efficiencies increase. In the β -CD/TX-100/TBZ (Fig. 1c, $\lambda_{ex}/\lambda_{em} = 308/484$ nm) system, the weak phosphorescence observed results from the formation of a 1 : 1 : 1 ternary inclusion complex, which strengthens the shielding effect and rigidity of the system, but lacks the induction effect of the heavy atom perturber. For the β -CD/TX-100/TBZ/KI (Fig. 1e, $\lambda_{ex}/\lambda_{em} = 298/481$ nm) system, a strong and stable phosphorescence is emitted, and the luminescence intensity increases compared with that reported in ref. 9 (Fig. 1d, $\lambda_{ex}/\lambda_{em} = 300/488$ nm), which indicates that the microenvironment around TBZ is improved. Great changes of the phosphorescence intensity of TBZ in the β -CD/TX-100/TBZ/KI system compared with other systems make us think that the ternary inclusion complex is formed,²³ which could affect the interaction between TBZ and KI.

3.2 Supramolecular multirecognition interaction between β -CD, TX-100 and TBZ

3.2.1 Synthesis and characterization of the β -CD/TBZ inclusion complex. According to the reaction routine reported in ref. 24, 0.01 mol TBZ was dissolved in a minimum volume of ethanol at 60 °C, and then 1.5 equiv. of β -CD aqueous solution was added dropwise with continuous, intensive stirring. The mixed solution was refluxed with vigorous agitation at 70 °C for about 4 h. Then it was heated to 80–82 °C to remove ethanol. After the mixture was cooled to room temperature, it was stirred for 8 h at ambient temperature. The reaction mixture was stored overnight at 4 °C, then centrifuged and filtered on a sintered glass filter. The product was obtained and washed sequentially with doubly distilled water and ethanol, then dried in a vacuum oven at an elevated temperature (60–65 °C). A 7.32 g white crystalline product was obtained at 60% yield; mp: 268–270 °C, $[\alpha]_D^{25} = -117.02^\circ \text{ mL dm}^{-1} \text{ g}^{-1}$ (determined in methanol/water (1 : 2) at 25 °C); R_f : 0.47 (GF254 silica gel plates, spread with acetic ether/dichloromethane (1 : 1), iodine vapor visualization).

Inclusion complex formation is proven by IR spectrometry because the absorbance peak of the included part of the guest molecule is generally shifted or its intensity altered.²⁵ By comparison of the IR spectra of TBZ (Fig. 2a), β -CD (Fig. 2b), the physical mixture of TBZ and β -CD (Fig. 2c), and the inclusion complex (Fig. 2d), it can be seen that the spectrum of d is essentially the combination of a and b, which indicates that the physical mixture can not lead to inclusion; there are

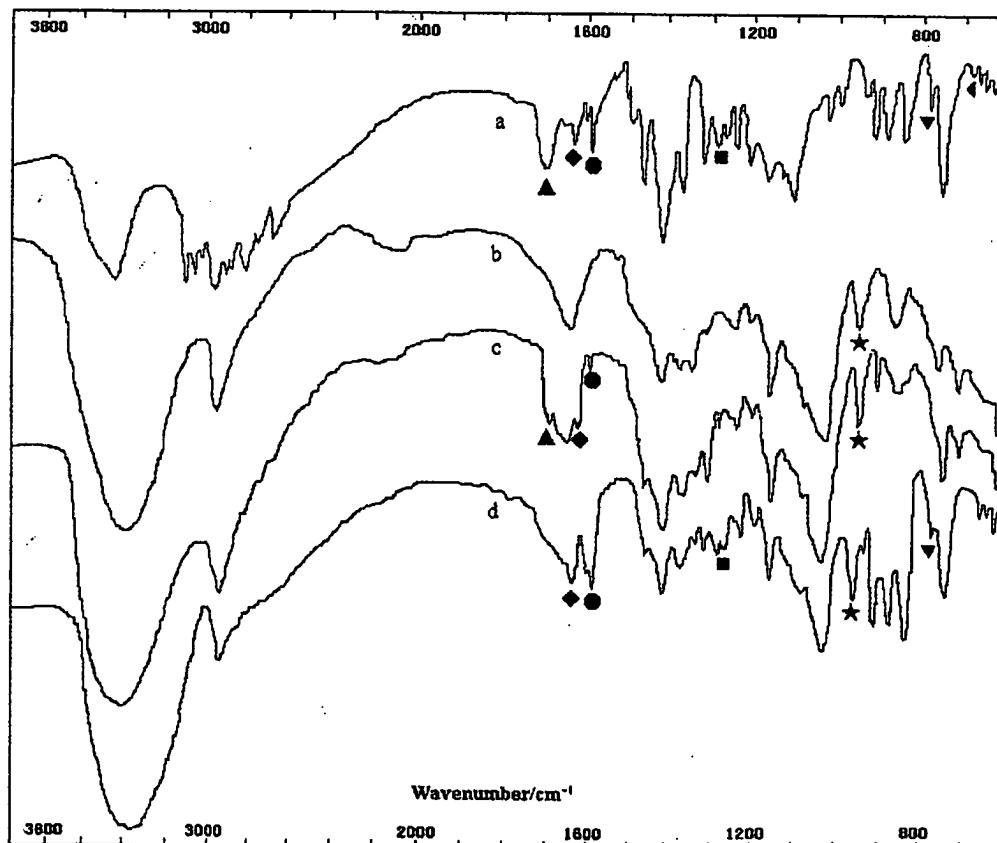


Fig. 2. Infrared spectra of TBZ, β -CD, their physical mixture, and the inclusion complex.

obvious differences between the spectra of c and d and some characteristic IR absorption peaks of TBZ and β -CD have changed in the inclusion complex: the $1028\text{--}1151\text{ cm}^{-1}$ absorption band in the inclusion complex assigned to the characteristic C-O-C bond antisymmetric stretching vibration and C-C/C-O bond stretching vibration of β -CD;²⁶ the 1300 cm^{-1} absorption peak (■), which can be assigned to the skeleton stretching vibration of the heterocycle, namely the thiazole ring, appears in a and d; the 951 cm^{-1} absorption peak (★) in b is due to the α -1,4- bond skeleton vibration of β -CD blue-shifted to 958 cm^{-1} in d; the benzene ring skeleton vibration absorption peak at 1580 cm^{-1} (●) and the benzene ring $-\text{C}=\text{C}-$ bond stretching vibration absorption peak at 1600 cm^{-1} (◆) in a red-shifting to 1570 cm^{-1} and 1585 cm^{-1} in d, respectively; the 770 cm^{-1} (▼) absorption peak assigned to four adjoining hydrogens at the benzene ring in a blue-shifting to 776 cm^{-1} in d; the 1691 cm^{-1} (▲) and 673 cm^{-1} (◀) absorption peaks in a disappeared in d. Based on these facts, it can be initially concluded that the benzene ring of TBZ has been included into the β -CD cavity to form a supramolecular inclusion complex.

3.2.2 Studies on the supramolecular interaction between β -CD and TX-100 by the surface tension method. As shown in Fig. 3, in the absence of β -CD, the surface tension of the solutions begins to decrease gradually with increasing concentration of TX-100, the critical micelle concentration (CMC) was $0.22 \times 10^{-3}\text{ mol L}^{-1}$. In the presence of β -CD, however, a continuous increase in β -CD concentration results in an increase in surface tension of the solutions. The addition of β -CD shifts the CMC* (apparent CMC) to higher concentrations (Table 1). This means that the higher β -CD concentration is used, and the higher TX-100 concentration is needed for micelle formation. It can also be found that when the concentration of TX-100 is CMC*, the surface tension of the solutions is equal to that of the solutions that contain TX-100 for the concentration of CMC in the absence of β -CD, which indicates that β -CD or the inclusion complex formed between β -CD and surfactant has no surface activity,²⁷ and there is no interaction occurring between the inclusion complex and micelles because the micelles can not enter the β -CD's cavity as a result of its

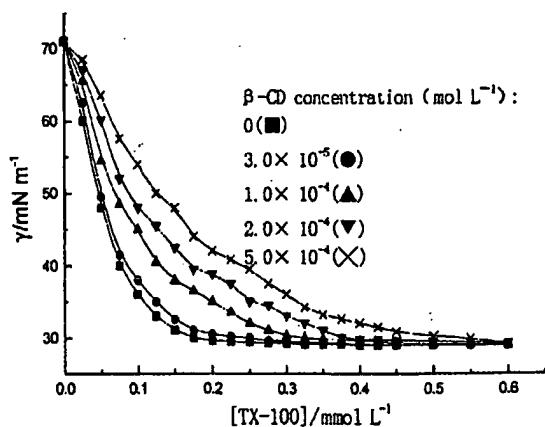


Fig. 3 Dependence of surface tension on the concentration of TX-100

Table 1 CMC* of TX-100 at different concentrations of β -CD

$[\beta\text{-CD}]\text{mmol L}^{-1}$	$\text{CMC}^*\text{TX-100}/\text{mmol L}^{-1}$
0.10	0.31
0.20	0.40
0.30	0.46
0.40	0.56
0.50	0.59
0.60	0.59

disadvantageous structure. The variation in the surface tension of the solution and the CMC indicates the occurrence of a supramolecular interaction between β -CD and TX-100.²⁸ The results show that it is the surfactant monomers, not the micelles, contributing to the lowering of the surface tension²⁹ and the formation of the inclusion complex.

The stoichiometry of the β -CD/TX-100 system is determined by the equation below:³⁰

$$1/\Gamma_i = 1/n + 1/\theta S_i n K_A$$

where Γ_i is $^bS_i/C$ (bS_i is referred to as the concentration difference value of TX-100 in the absence and presence of β -CD when the surface tension is fixed), C is the concentration of β -CD added, 0S_i is the free concentration of TX-100 in the absence of β -CD, n is the number of guest molecules included into the β -CD's cavity, and K_A is the equilibrium constant. Fig. 4 shows a plot of $1/\Gamma_i$ vs. $1/\theta S_i$, it can be seen that there is a linear relationship between $1/\Gamma_i$ and $1/\theta S_i$. The value of n can be obtained from the intercept and K_A can be evaluated from the ratio of the intercept to slope, and the average values of K_A and n are listed in Table 2. The absorption data confirms the formation of a 1 : 1 β -CD/TX-100 inclusion complex and K_A is determined to be $(8.08 \pm 0.14) \times 10^3\text{ L mol}^{-1}$.

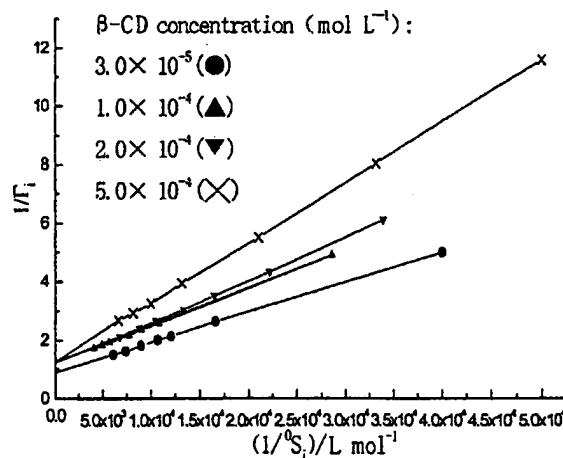
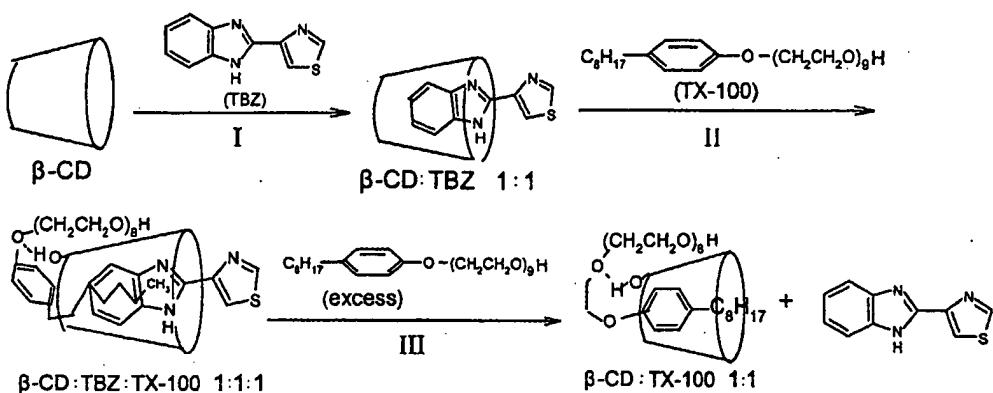


Fig. 4 Plot of $1/\Gamma_i$ versus $1/\theta S_i$.

Table 2 Average equilibrium constant and inclusion ratio of the β -CD-TX-100 complex

$[\beta\text{-CD}]\text{mmol L}^{-1}$	$K_A/\text{L mol}^{-1}$	n
0.03	$(9.11 \pm 0.12) \times 10^3$	0.94
0.10	$(8.56 \pm 0.13) \times 10^3$	1.13
0.20	$(7.52 \pm 0.21) \times 10^3$	1.15
0.50	$(7.13 \pm 0.10) \times 10^3$	1.12
Average	$(8.08 \pm 0.14) \times 10^3$	1.08



Scheme 3 Formation process of the ternary inclusion complex.

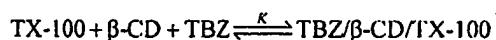
In conclusion, we believe that the ternary inclusion complex is formed.

3.2.3 Determination of apparent association constant of the ternary inclusion complex. Based on the results of section 3.2.2, we assume that the formation process of the ternary inclusion complex is as shown in Scheme 3:

The equilibrium shifting method³¹ was used to evaluate the stoichiometry of the β -CD/TBZ/TX-100 ternary complex. As

is shown in Fig. 5, the slope of the two plots is 1.14 and 1.10, which suggests the formation of a 1 : 1 : 1 ternary complex.

The formation of the ternary inclusion complex can be described by the following equation:



The 1 : 1 : 1 stoichiometry of the β -CD/TBZ/TX-100 system is also confirmed by the Benesi-Hildebrand equation:³²

$$1/\Delta I_p = 1/\alpha K[S][\beta\text{-CD}] + 1/\alpha$$

where K is the apparent association constant and ΔI_p is the difference in the phosphorescence intensity of TBZ in the presence and absence of β -CD. $[S]$ and $[\beta\text{-CD}]$ is the equilibrium concentration of TX-100 and β -CD, respectively. α is a combined instrumental constant. When the initial concentration of β -CD, $C_{\beta\text{-CD}}$, is much greater than that of the inclusion complex, $[\beta\text{-CD}]$ can be replaced by $C_{\beta\text{-CD}}$. Fig. 6 shows a representative double-reciprocal plot of $1/\Delta I_p$ vs. $1/[\beta\text{-CD}]$ for the TBZ/ β -CD/TX-100 system. As can be seen, a good linear relationship is observed with a correlation coefficient of 0.9905, which confirms a 1 : 1 : 1 stoichiometry of a ternary inclusion complex in aqueous solution. The value of K evaluated from the ratio of the intercept to slope is $(3.42 \pm 0.23) \times 10^6 \text{ L}^2 \text{ mol}^{-2}$.

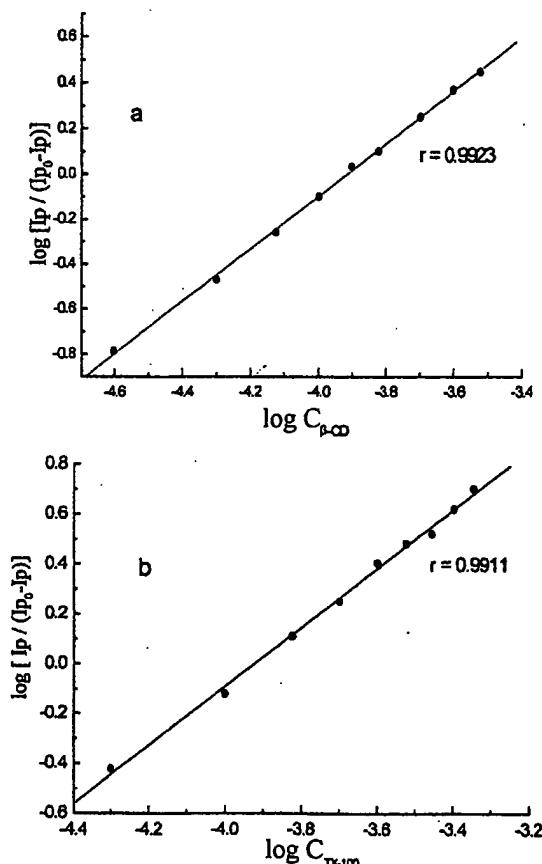
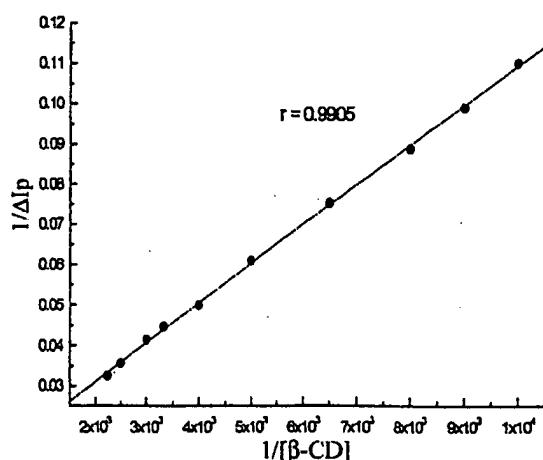


Fig. 5 Equilibrium shifting method for the determination of formation ratio of the ternary inclusion complex. $C_{\text{TBZ}} = 200 \mu\text{g L}^{-1}$, $C_{\text{K1}} = 0.20 \text{ mol L}^{-1}$, (a) $C_{\text{TX-100}} = 4.0 \times 10^{-4} \text{ mol L}^{-1}$, (b)



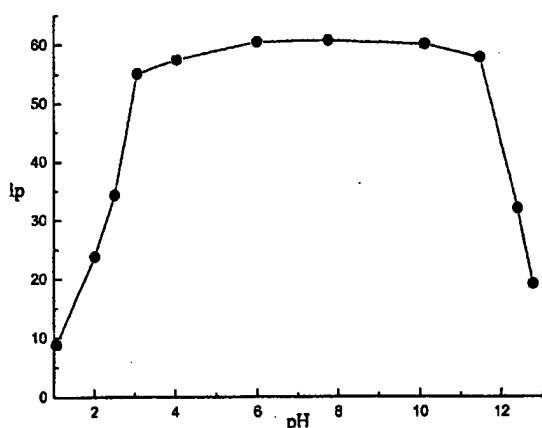


Fig. 7 Dependence of RTP on pH. $C_{TBZ} = 200 \mu\text{g L}^{-1}$, $C_{KI} = 0.20 \text{ mol L}^{-1}$, $C_{\beta\text{-CD}} = 5.0 \times 10^{-4} \text{ mol L}^{-1}$, $C_{TX-100} = 6.0 \times 10^{-4} \text{ mol L}^{-1}$.

3.3 Optimization of manifold parameters

3.3.1 Effect of pH. Fig. 7 shows the dependence of phosphorescence intensity on pH. It is not necessary to eliminate oxygen in this ternary system. The phosphorescence intensity reaches its maximum and remains constant in the pH range 2.5–11.5, which is greatly broadened compared with the method using a chemical oxygen scavenger⁹ (6.5–10). Thus, a sodium citrate–sodium hydroxide buffer solution is used to adjust at pH 5.50. When the pH is lower than 2.5, large quantities of onium salts are formed between TX-100 and protons,³³ which increases the hydrophilic ability of TX-100. This increases the water quantity contained in the micellar interior layers, and ultimately increases the quenching efficiencies of the dissolved oxygen. When the pH exceeds 11.5, TBZ exists in the form of an anion,³⁴ which delocalizes the conjugated system. Therefore, perhaps a certain kind of excimer is formed between TBZ and TX-100, the energy of the excited state is delivered from the excimer to the solvent along the hydrocarbon chain of TX-100 to decay the phosphorescence.

3.3.2 Effect of KI concentration. The ether oxygen atoms of TX-100 have a similar interacting behavior to the oxygen atoms of crown ethers, and can coordinate with K^+ to make part of the micelle present positive electricity. Therefore, I^- is enriched zonally by electrostatic attraction to decrease the distance between TBZ and I^- and to increase drastically their interacting probabilities. Compared with the reported HAI-RTP method,⁹ the KI concentration decreases dramatically to 0.15–0.45 mol L⁻¹, therefore 0.20 mol L⁻¹ is used. Because the heavy atom perturber can increase the speed of the $\text{S}_1 \rightarrow \text{T}_1$ intersystem transition and the speed of the radiative transition of the triplet state simultaneously to shorten the phosphorescence lifetime,³⁵ an evaluation of the phosphorescence lifetime of TBZ in the present experiment was carried out. Usually the attenuation of phosphorescence intensity follows the equation:³⁵ $\ln I_0 - \ln I_t = -t/\tau$, where I_0 and I_t referred to the phosphorescence intensity when the time is zero and t , respectively. Different I values were measured at different times, then the plot $\ln I_t$ vs. t was made. The phosphorescence

experimental conditions, the average value of τ was determined from 5 measurements and was found to be 0.8 ms; an increase of 9 times compared with that reported in the literature.⁹

3.3.3 Effect of $\beta\text{-CD}$ concentration. Studies on the dependence of RTP on the $\beta\text{-CD}$ concentration were performed. In the concentration range $3.0 \times 10^{-4} \text{ mol L}^{-1}$ – $8.0 \times 10^{-4} \text{ mol L}^{-1}$, RTP is the highest and remains constant. Therefore $5.0 \times 10^{-4} \text{ mol L}^{-1}$ $\beta\text{-CD}$ is used.

3.3.4 Effect of TX-100 concentration. The influence of TX-100 concentration was studied. It can be observed that in the TX-100 concentration range $6.0 \times 10^{-4} \text{ mol L}^{-1}$ – $6.3 \times 10^{-4} \text{ mol L}^{-1}$, RTP is the highest and remains constant. When the concentration exceeds $6.3 \times 10^{-4} \text{ mol L}^{-1}$, RTP begins to gradually decrease, therefore $6.0 \times 10^{-4} \text{ mol L}^{-1}$ TX-100 is fixed. It is found that the optimum concentration of TX-100 ($6.0 \times 10^{-4} \text{ mol L}^{-1}$) is a bit higher than the CMC* ($5.9 \times 10^{-4} \text{ mol L}^{-1}$, Table 1) in the presence of $5.0 \times 10^{-4} \text{ mol L}^{-1}$ $\beta\text{-CD}$, which indicates that there are two forms of TX-100 coexisting in the solution: monomers and micelles. The monomers act as the spacial filler and regulator, as well as the hydrogen bond forming agents in the formation of the ternary inclusion complex. The micelles aggregate around the inclusion complex to provide a more hydrophobic micro-environment for the complex through its hydrophobic interior layers. When the TX-100 concentration is higher than a certain value ($6.3 \times 10^{-4} \text{ mol L}^{-1}$ in this reaction condition), more and more TX-100 molecules are driven by the hydrophobic force to compete with TBZ for the $\beta\text{-CD}$'s cavity. The final results are that the TBZ molecules are expelled from the $\beta\text{-CD}$'s cavity and are dissolved in a mixed solution with large amount of micelles and $\beta\text{-CD}$, which is extremely different from the microenvironment experienced in the ternary inclusion complex. Because the shielding protection for the triplet state of TBZ has been removed, the intermolecular collision is reinforced, and the rate of nonradiative decay process is increased, which leads to the drastic lowering of phosphorescence quantum yield and the occurrence of phosphorescence quenching.

3.4 Analytical parameters

The RTP emission of TBZ produced through the ternary inclusion complex formation might be very useful from an analytical point of view. Therefore, room temperature phosphorimetry for the determination of TBZ in bulk aqueous solution in the presence of $\beta\text{-CD}$ and TX-100 was developed.

Into a series of 10 mL colorimetric tubes was added TBZ standard solution. Under optimum conditions, the RTP intensity was measured. The results show that the RTP intensity is linear over a TBZ concentration range of 20–820 ng mL⁻¹. The linear regression equation is $I_p = 5.21 \times 10^{-2}c (\text{ng mL}^{-1}) + 54.4$ ($r = 0.9937$). The relative standard deviation (RSD) is 1.9% obtained from a series of 11 standards each containing 200 $\mu\text{g L}^{-1}$ TBZ. Based on the definition by IUPAC,³⁶ $C_L = KS_0/S$, where C_L is the limit of detection, K is a constant combined with the confidence level, S_0 (0.037) is

Table 3 Comparison with other methods for the determination of thiabendazole

Methods	Detection limits	Linear range	Reference
HPLC	0.1 $\mu\text{g mL}^{-1}$	—	5
LC-MS	—	2–130 $\mu\text{g mL}^{-1}$	6
Fluorimetry	0.29 ng mL^{-1}	5–40 ng mL^{-1}	7
Capillary electrophoresis-mass spectrometry	33 ng mL^{-1}	0.2–5 $\mu\text{g mL}^{-1}$	8
RTP	7.7 ng mL^{-1}	15.4–150 ng mL^{-1}	9
MEKC	0.1 $\mu\text{g mL}^{-1}$	0.5–2.3 $\mu\text{g mL}^{-1}$	10
Capillary electrophoresis-electrospray mass spectrometry	0.01 $\mu\text{g mL}^{-1}$	1–10 $\mu\text{g mL}^{-1}$	11
CZE	0.13 $\mu\text{g mL}^{-1}$	2.5–10 $\mu\text{g mL}^{-1}$	12
LC-ESI-MS-MS	2 ng mL^{-1}	7–240 ng mL^{-1}	13
RTP	2.1 ng mL^{-1}	20–820 ng mL^{-1}	This work

Table 4 Effect of foreign substances on the determination of 200 $\mu\text{g L}^{-1}$ TBZ

Tolerance ratio in mass	Foreign ions
6000	Na^+ , NH_4^+ , K^+ , Ba^{2+} , Ca^{2+} , Zn^{2+} , B^{3+} , Cl^- , NO_3^- , CN^- , Br^- , H_2PO_4^- , ClO_4^- , CO_3^{2-} , SO_4^{2-} , PO_4^{3-} , NaCl
3500	Sucrose, lactose, glucose, glycine, acetate acid
2500	Ni^{2+} , Cu^{2+} , Mn^{2+} , boracic acid, gelatin, starch, Na_2EDTA , sorbitol, ethanol, gum acacia powder, sodium acetate, mannitol
500	Be^{2+} , Co^{2+} , NO_2^- , Ac^-
80	Cd^{2+} , $\text{Cr}_2\text{O}_7^{2-}$, methyl cellulose
30	Pb^{2+} , Fe^{2+} , MnO_4^-
25	Fe^{3+} , Al^{3+}

solutions, and S is the slope of the standard curve. When the confidence level is 90%, the limit of detection of the proposed method is determined to be 2.1 ng mL^{-1} . The proposed method was compared to the previously reported methods.^{5–13} The results show that the proposed method has good sensitivity in a much wider linear range.

3.5 Effect of foreign interference

A systematic study was carried out on the effects of foreign interferences on the determination of 200 $\mu\text{g L}^{-1}$ TBZ. A 7000-fold mass excess of each interference over TBZ was tested first, if interferences occurred, the ratio was reduced gradually until the interferences ceased. The criterion for interference was fixed at a $\pm 5\%$ variation of the average RTP intensity calculated for the established level of TBZ. The results are shown in Table 4, which shows that the proposed method has excellent selectivity.

3.6 Applications

To evaluate the accuracy of the proposed method, a standard adding method was performed to detect trace amounts of TBZ in tap water, Da Ming Lake water and fresh pineapple. The samples' treatment was done according to the following procedures.^{37,38} 500 mL tap water or Da Ming Lake water was filtered through qualitative filter paper with pore diameter of 0.45 μm to remove the undissolving substance and collected in a polyethylene container that had been carefully cleaned with nitric acid. A certain quantity of TBZ standard solution was transferred into a 100 mL volumetric flask, then diluted to the mark with the filtered tap water or Da Ming Lake water.

Table 5 Results for the recoveries of TBZ from real samples

Sample	TBZ added/ ng mL^{-1}	TBZ found/ ng mL^{-1} ($n = 5$)	Recovery (%)	RSD (%)
Tap water	40.0	40.8	102	2.4
	100	95.0	95	1.6
	120	116	97	1.8
Lake water	200	195	98	2.3
	400	408	102	2.5
	80.0	78.2	98	3.1
Pineapple	60.0	57.1	95	2.9
	130	129	99	2.1
	150	152	101	2.4

of syrup and a certain quantity of TBZ were sequentially added into a 100 mL beaker. The samples were extracted twice with 20 mL ethyl acetate, filtered under vacuum, and the residues washed with 10 mL ethyl acetate. Both extracts were mixed and dried in a rotary evaporator at 40 °C. The obtained solid was solubilized with 10 mL acetonitrile and transferred into a 100 mL volumetric flask, then diluted to the mark with doubly distilled water. The TBZ content was calculated according to the linear regression equation. The results are shown in Table 5. As can be seen, the precision and accuracy of the proposed method are satisfactory.

4. Conclusions

A strong and stable room temperature phosphorescence signal resulting from a 1 : 1 : 1 β -cyclodextrin/thiabendazole/triton X-100 supramolecular ternary inclusion complex can be induced by KI as a heavy atom perturber without removing dissolved oxygen from the solution. The formation of the inclusion complex protects the phosphorescence against varying quenching factors, drastically decreasing the concentration of heavy atom perturber and optimizing the experimental conditions. A phosphorescent method with high sensitivity and selectivity for the determination of TBZ in the bulk aqueous solution has been developed. The proposed method was successfully applied to determine trace amounts of TBZ in real samples.

Acknowledgements

This work was supported by the Main National Nature Science Foundation of China (No. 20335030) and the Main Nature Science Foundation of Shandong Province, China

Bo Tang,* Xu Wang, Guangli Wang, Yan Wang and Zhenzhen Chen
College of Chemistry, Chemical Engineering and Materials Science,
Shandong Normal University, Jinan, 250014, China.
E-mail: tangb@sdnu.edu.cn; Fax: +86-531-86180017;
Tel: +86-531-86180010

References

- 1 C. Q. Xin and J. Y. You, *New Edition of Pharmacology*, People's Medical Publishing House, Beijing, 12th edn., 1985, p. 176.
- 2 T. Takana, *Food Addit. Contam.*, 2001, **18**, 375-383.
- 3 D. L. Zhang and P. C. Quantic, *Acta Hortic.*, 2000, **517**, 427-433.
- 4 H. O. Zawa, M. Hirokado, A. Ibe, Y. Shimamura, K. Nakajima, K. Kimura and K. Yasuka, *Tokyo-toritsu Eisei Kenkyusho Kenkyu Nenpo*, 1999, **50**, 119-123.
- 5 A. D. Muccio, S. Girolimetti, D. A. Barbini, P. Pelosi, T. Generali, L. Vergori, G. D. Merulisi, A. Leonelli and P. Stefanilli, *J. Chromatogr. A*, 1999, **833**, 61-65.
- 6 M. S. Young, M. F. Early, C. R. Mallet and J. Krol, *J. AOAC Int.*, 2001, **84**, 1608-1613.
- 7 Z. D. Picon, G. M. Martinez, F. A. Garrido and J. L. Vidal, *Analyst*, 2000, **125**, 1167-1174.
- 8 S. Takeda, K. Fukushima, K. Chayama, Y. Nakayama, Y. Tanaka and S. Wakida, *J. Chromatogr. A*, 2004, **1051**, 297-301.
- 9 A. Segura-Carretero, C. C. Blanco, B. C. Diaz, J. F. Fernández-Sánchez and A. Fernández-Gutiérrez, *Anal. Chim. Acta*, 2000, **417**, 19-30.
- 10 R. Rodríguez, Y. Picó, G. Font and J. Mañes, *J. Chromatogr. A*, 2001, **924**, 387-396.
- 11 R. Rodríguez, Y. Picó, G. Font and J. Mañes, *J. Chromatogr. A*, 2002, **949**, 359-366.
- 12 R. Rodríguez, I. Boyer, G. Font and Y. Picó, *Analyst*, 2001, **126**, 2134-2138.
- 13 K. Granby, J. H. Andersen and H. B. Christensen, *Anal. Chim. Acta*, 2004, **520**, 165-176.
- 14 J. A. Arancibia and G. M. Escandar, *Analyst*, 1999, **124**, 1833-1838.
- 15 D. C. Bibby, N. M. Davies and G. T. Ian, *Int. J. Pharm.*, 2000, **197**, 1-11.
- 16 R. H. Frömming and J. Szejtli, *Cyclodextrin in Pharmacy*, Dordrecht, Kluwer, 1994, pp. 105-115.
- 17 Y. B. Jiang, J. G. Xu and G. Z. Chen, *Acta. Chim. Sin.*, 1991, **49**, 850-854.
- 18 A. E. Kaifer, *Adv. Supramol. Chem.*, 1992, **2**, 1-24.
- 19 M. D. Veiga and F. Ahsan, *Eur. J. Pharm. Sci.*, 2000, **9**, 291-299.
- 20 L. D. Li, X. K. Chen, L. Mou, W. Q. Long and A. J. Tong, *Anal. Chim. Acta*, 2000, **417**, 177-183.
- 21 A. Seguracarretero, C. Crucesblanco, J. F. Fernandez Sanchez, B. Canabatediaz and A. J. Fernandezgutierrez, *J. Agric. Food Chem.*, 2000, **48**, 4453-4459.
- 22 Z. K. Zhou, T. R. Gu and J. M. Ma, *Base of Colloid Chemistry*, Beijing University Press, Beijing, 1996, 2nd edn., p. 26.
- 23 X. Z. Du, Y. Zhang, Y. B. Jiang, L. R. Lin, X. Z. Huang and G. Z. Chen, *Acta. Chim. Sin.*, 1998, **56**, 453-458.
- 24 L. Szente, *Comprehensive of Supramolecular Chemistry*, ed. J. L. Atwood and J.-M. Lehn, Pergamon, Oxford, 1996, vol 3: Cyclodextrins, ch. 7, pp. 243-251.
- 25 L. Szente, *Analytical methods for cyclodextrins, cyclodextrin derivatives and cyclodextrin complex*, in *Comprehensive Supramolecular Chemistry*, Pergamon Press, Oxford, 1996, vol. 3, pp. 253-278.
- 26 L. H. Tong, *Chemistry of Cyclodextrin-Foundation and Application*, Science Press, Beijing, 1st edn., 2001, p. 215.
- 27 U. R. Dharmawardana, S. D. Christian, E. E. Tucker, R. W. Taylor and J. F. Scamehorn, *Langmuir*, 1993, **9**, 2258-2263.
- 28 Y. Nemoto and H. Funahashi, *J. Colloid Interface Sci.*, 1981, **79**, 313-318.
- 29 N. Funasaki, *Bull. Chem. Soc. Jpn.*, 1992, **65**, 1323-1330.
- 30 Y. Z. Hui and J. H. Gu, *Acta Chim. Sin.*, 1981, **39**, 309-317.
- 31 Z. M. Luo, *Chin. Chem. Soc.*, 1987, **49**-51.
- 32 S. Hamai, *J. Am. Chem. Soc.*, 1989, **111**, 3954.
- 33 P. Becher, *J. Colloid Sci.*, 1962, **17**, 801.
- 34 P. C. Tway and L. C. Love, *J. Phys. Chem.*, 1982, **86**, 5227-5230.
- 35 G. G. Giachino, *J. Chem. Phys.*, 1970, **52**, 2964.
- 36 H. M. Irving, H. Freiser and T. S. West, *IUPAC. Compendium of Analytical Nomenclature, Definitive Rules*, Pergamon Press, Oxford, 1978.
- 37 F. Capitán, E. Alonso, R. Avidad, L. F. Capitán-Vallvey and J. L. Vilchez, *Anal. Chem.*, 1993, **65**, 1336-1339.
- 38 A. S. Carretero, C. C. Blanco, R. E. Fernandez and A. F. Gutierrez, *J. Anal. Chem.*, 1998, **360**, 605-608.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

BLACK BORDERS

IMAGE CUT OFF AT TOP, BOTTOM OR SIDES

FADED TEXT OR DRAWING

BLURRED OR ILLEGIBLE TEXT OR DRAWING

SKEWED/SLANTED IMAGES

COLOR OR BLACK AND WHITE PHOTOGRAPHS

GRAY SCALE DOCUMENTS

LINES OR MARKS ON ORIGINAL DOCUMENT

REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY

OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.